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Validation of two tropical marine bivalves as bioindicators of mining contamination in the New Caledonia lagoon: Field transplantation experiments

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28 **ABSTRACT.** The bioaccumulation and retention capacities of some key local
29 contaminants of the New Caledonia lagoon (Ag, As, Cd, Co, Cr, Cu, Mn, Ni and Zn)
30 have been determined in the oyster *Isognomon isognomon* and the edible clam
31 *Gafrarium tumidum* during transplantation experiments. In a first set of experiments,
32 oysters and clams from a clean site were transplanted into contaminated sites. Uptake
33 kinetics determined in the field indicated that for Cr and Cu in oysters and Co, Ni, and
34 Zn in clams, concentrations in transplanted bivalves reached those of resident
35 organisms after 100d, whereas for the other elements, it would require a longer time
36 for transplanted bivalves to reach the same levels as in the resident populations (e.g.,
37 up to 3 years for Cd). However, the slow uptake rate for metals observed in the latter
38 transplantation is rather related to low bioavailability of metals at the contaminated
39 sites than to low bioaccumulation efficiency of the organisms. Indeed, results of a
40 second transplantation experiment into two highly contaminated stations indicated a
41 faster bioaccumulation of metals in both bivalves. Results of both transplantations
42 point out that the clam *G. tumidum* is a more effective bioindicator of mining
43 contamination than *I. isognomon*, since it is able to bioaccumulate the contaminants to
44 a greater extent. However the very efficient metal retention capacity noted for most
45 elements indicates that organisms originating from contaminated sites would not be
46 suitable for monitoring areas of lower contamination. Hence, geographical origin of
47 animals to be transplanted in a monitoring perspective should be carefully selected.

48

49 **Keywords:** Molluscs, Oyster, Clam, Bioaccumulation, Biomonitoring, Metals

50

51 **I. Introduction**

52 New Caledonia is a small South Pacific island whose main economic resources are
53 derived from nickel exploitation. Among other, local mining activities result in large
54 anthropogenic inputs of metals into the SW lagoon and thereby constitute a potential
55 threat to the local coastal marine ecosystems (e.g., Bird et al. 1984) but it is only
56 recently that relevant information was made available regarding levels of metal
57 contamination and their possible impacts on the local marine ecosystems (e.g.,
58 Hédouin et al. 2009, Metian et al. 2008a). Therefore monitoring of environmental
59 contamination originating from mining activities in the lagoon still needs some
60 significant scientific inputs.

61 Among the common approaches used to study environmental contamination, the use
62 of bivalve molluscs as bioindicator species has proved to be a valuable and
63 informative technique (e.g., Mussel Watch, Goldberg et al. 1983). This approach has
64 been particularly developed in temperate areas, whereas in sub-tropical and tropical
65 areas the scarcity of available information makes the identification of species that
66 could be used as suitable bioindicators difficult (e.g., Phillips 1991). However, the
67 screening of metal concentrations in a variety of marine organisms from several parts
68 of the SW lagoon of New Caledonia has identified the oyster *Isognomon isognomon*,
69 the edible clam *Gafrarium tumidum*, and the alga *Lobophora variegata* as potential
70 bioindicators (Hédouin et al. 2009). It has been shown recently that the alga *L.*
71 *variegata* was an efficient bioindicator of metals in seawater in both controlled and *in*
72 *situ* conditions (Hédouin et al. 2008, Metian et al. 2008b). In addition, recent
73 experimental works on the oyster *I. isognomon* and the clam *G. tumidum* have
74 indicated that these two species bioconcentrate and efficiently retain several elements
75 when exposed via seawater, sediments or their food (Hédouin et al. 2010b). More

76 importantly, both bivalve species were shown to concentrate As, Cd, Co, Cr, Mn, Ni,
77 and Zn in direct proportion to their concentrations in seawater and food (Hédouin et
78 al. 2007, 2010b).

79 Although the former experiments were carried out under controlled conditions
80 simulating as closely as possible those in the natural environment, laboratory
81 experiments cannot reproduce exactly the conditions in the field. In this respect *in situ*
82 experiments offer a more ecologically-realistic approach, since they encompass all the
83 factors that actually occur in the field and may possibly interfere with or influence
84 bioaccumulation processes (e.g., Cain and Luoma 1985, Hédouin et al. 2008).

85 Active biomonitoring using transplantation of organisms from one site to another is a
86 very efficient way to follow the degree of contamination at various sites (e.g. Hédouin
87 et al. 2008). The main advantages over the traditional passive biomonitoring (viz.
88 monitoring of metal concentrations using resident natural populations) are that (1) the
89 sites to monitor may be chosen independently of the presence of natural populations
90 and (2) the influence of external and internal factors (e.g. seasonal variation, size or
91 age) susceptible to induce bias in data comparison is reduced (Phillips and Rainbow
92 1993).

93 The aim of the present field study was to determine the relevance of using the oyster
94 *I. isognomon* and the clam *G. tumidum* as bioindicator species of metal contamination
95 in tropical waters. Through two different field transplantation experiments, the ability
96 of both species to bioaccumulate and depurate 9 selected elements (Ag, As, Cd, Co,
97 Cr Cu, Mn, Ni and Zn) under natural conditions has been assessed as well as their
98 ability to inform about the contamination status of their surrounding environment. A
99 power analysis was also carried out to determine the sample size required to allow
100 differentiating among realistic field contamination levels.

101 **II. Materials and methods**

102 Between March and June 2005, two series of transplantation experiments were
103 performed in New Caledonia using the oyster *Isognomon isognomon* and the clam
104 *Gafrarium tumidum*. Based on previous field results (Hédouin et al. 2009) sampling
105 stations were selected according to their apparent degree of metal contamination. Maa
106 Bay (subtidal station for oysters) and Ouano Beach (intertidal station for clams) were
107 identified as clean stations with low element concentrations in bivalve tissues and
108 sediments for all elements except As. In contrast, Boulari Bay (for oysters) and
109 Grande Rade -GR_{Int}- (intertidal station for clams) were designated as highly
110 contaminated stations (Fig. 1).

111 ***II.1. Experimental design***

112 Since body size is well known to affect metal concentrations in marine invertebrates
113 (e.g. Boyden 1977), only individuals with shell length longer than 70 mm for *I.*
114 *isognomon* (Metian 2003) and shell width greater than 35 mm for *G. tumidum*
115 (Hédouin et al. 2006) were considered in order to minimize size-related variability.
116 Two types of transplantations were conducted. A first reciprocal transplantation
117 aimed at assessing metal bioaccumulation and depuration processes in natural
118 populations living in two contrasted environmental conditions (see Fig. 2). A second
119 transplantation was conducted to test the ability of both selected species to inform
120 about the contamination level of their surrounding environment in a heavily polluted
121 area (unidirectional transplantation in Grande Rade; Fig. 2).

122 ***II.1.1. Experiment 1: Reciprocal transplantations***

123 Eighty oysters and 80 clams were collected from the two selected clean stations, Maa
124 Bay and Ouano Beach, respectively. A sub-sample of 10 organisms from each station

125 was used for determination of baseline concentrations of the 9 selected elements (Ag,
126 As, Cd, Co, Cr Cu, Mn, Ni and Zn) at the beginning of the experiment. The remaining
127 oysters and clams (n = 70 per species) were transplanted for 100 d to the heavily
128 contaminated stations, Boulari Bay and Grande Rade, (GR_{Int}, intertidal station),
129 respectively. The reciprocal transplantation was undertaken with another batch of 80
130 oysters and 80 clams collected in Boulari Bay and Grande Rade (GR_{Int}), respectively,
131 and transplanted to the clean stations, Maa Bay (for oysters) or Ouano Beach (for
132 clams).

133 Organisms (transplanted and control resident individuals) at each station were placed
134 in plastic mesh cages (60 × 60 cm; 2-cm mesh size), which allowed free exchange of
135 seawater. The plastic cages containing the oysters were placed at 5 m depth, which
136 corresponds to their natural habitat; those with clams were fixed in an intertidal
137 position and inserted within the sediments in order to reproduce to the best the living
138 condition of the clams. In order to monitor possible natural variation in element
139 concentrations at the different stations, resident organisms (n = 5 per species) and
140 superficial sediments (top 3-cm layer) were sampled simultaneously with the
141 transplanted organisms (n = 7) from clean and contaminated stations at different
142 times. Oysters were collected by SCUBA diving and the clams by hand picking at low
143 tide.

144 *II.1.2. Experiment 2: Unidirectional transplantation in Grande* 145 *Rade*

146 Grande Rade is locally influenced by anthropogenic inputs from the ‘Société Le
147 Nickel’ (SLN), a nickel processing plant. Two stations (GR₁ and GR₂) were chosen in
148 Grande Rade for this experiment because they had different levels of metal
149 contamination (Migon et al. 2007). GR₁ station is a highly polluted site due to its

150 proximity to the off-loading wharf of the SLN, whereas the second station GR₂, on the
151 opposite side of the Rade just in front of the SLN factory, is less contaminated than
152 GR₁ (Fig. 1).

153 The bivalves *I. isognomon* and *G. tumidum* (n = 140 per species) were collected from
154 the clean stations Maa Bay and Ouano Beach, respectively. Twenty organisms were
155 used for element analyses in order to establish the baseline concentrations of elements
156 at day 0 of transplantation; the remaining organisms (n = 120 per species) were
157 transplanted for 69 d into the two stations in Grande Rade (GR₁ and GR₂, n = 60 per
158 station per species) and held in 60 × 60 cm plastic cages (2-cm mesh size) immersed
159 at 5 m depth for both clams and oysters. Transplanted organisms (n = 30 per species)
160 in GR₁ and GR₂, and resident organisms (n = 20) from the clean stations (Maa Bay for
161 oysters and Ouano Beach for clams) were collected by SCUBA diving after 35 and 69
162 d. Sediment samples (top 3-cm layer) were collected simultaneously with organisms
163 from the clean and transplantation sites.

164 ***II.1. Sampling preparation and analyses***

165 Back to the laboratory, the bivalves were kept for 24 h in 30 l seawater from the same
166 sampling station to allow depuration of gut contents and of particulate material
167 present in the mantle cavity. Soft tissues were removed from the shells and were
168 weighed (wet weight; wwt), dried at 60°C until constant weight, and weighed again
169 (dry weight; dwt). They were then stored in acid-washed, hermetically sealed plastic
170 containers until analysis.

171 Sediments were similarly stored in acid-washed, hermetically sealed plastic bags and
172 frozen at -20°C. Sediments were then dried at 60°C for 5 d. In order to eliminate

173 heterogeneous materials (e.g., stones, fragment of corals), sediments were sieved (1-
174 mm mesh size) prior to analysis.

175 Aliquots of the biological samples (300 to 500 mg dwt) and sediment samples (300
176 mg dwt) were digested using a 3:1 (v:v) nitric-hydrochloric acid mixture (65%
177 suprapur HNO₃ and 30% suprapur HCl, Merck). Acid digestion of the samples was
178 carried out overnight at room temperature. Samples were then mineralized using a
179 CEM Corp. MARS 5 microwave oven (30 min with constantly increasing temperature
180 up to 100°C for sediments and 115°C for biological material, then 15 min at these
181 maximal temperatures). Each sample was subsequently diluted with milli-Q water
182 according to the amount of sample digested (10 ml / 100 mg).

183 Elements were analyzed using a Varian Vista-Pro ICP-OES (As, Cr, Cu, Mn, Ni, and
184 Zn) or a Varian ICP-MS Ultra Mass 700 (Ag, Cd and Co). Three control samples (two
185 Certified Reference Materials - CRM - and one blank) treated and analyzed in the
186 same way as the samples were included in each analytical batch. The CRM were
187 dogfish liver DOLT-3 and lobster hepatopancreas TORT-2 (NRCC). The results for
188 CRM indicated recoveries of the elements ranging from 81 % (Ni) to 113 % (Zn)
189 (Table 1). The detection limits were 31.0 (As), 1.3 (Cr), 3.8 (Cu), 0.15 (Mn), 1.1 (Ni)
190 and 2.4 (Zn) µg g⁻¹ dwt for ICP-OES and 0.1 (Ag), 0.15 (Cd) and 0.1 (Co) µg g⁻¹ dwt
191 for ICP-MS. All element concentrations are given on a dry weight basis (µg g⁻¹ dwt).

192 ***II.2. Data treatment and statistical analyses***

193 The uptake kinetics of the elements examined were described using either a simple
194 linear regression model (eq. 1) or a saturation exponential model (eq. 2):

$$195 \quad C_t = C_0 + k_u t \text{ (eq. 1)}$$

$$196 \quad C_t = C_0 + C_1 (1 - e^{-k_e t}) \text{ (eq. 2)}$$

197 where C_t and C_0 are the element concentrations in organisms at time t (d) and 0,
198 respectively ($\mu\text{g g}^{-1}$); $C_1 + C_0$ is the concentrations at steady state (C_{ss} ; $\mu\text{g g}^{-1}$); k_u is
199 the uptake rate constant ($\mu\text{g g}^{-1} \text{d}^{-1}$) and k_e is the depuration rate constant (d^{-1})
200 (Whicker and Schultz 1982).

201 Depuration kinetics of elements was described by either a simple linear regression
202 model (eq. 3) or a single-component exponential equation (eq. 4):

203
$$C_t = C_0 - k_e t \text{ (eq. 3)}$$

204
$$C_t = C_0 e^{-k_e t} + A \text{ (eq. 4)}$$

205 where A is a constant ($\mu\text{g g}^{-1}$).

206 Model constants and their statistics were estimated by iterative adjustment of the
207 model and Hessian matrix computation using the nonlinear curve-fitting routines in
208 the StatisticaTM software 5.2.1.

209 Element concentrations of sediments and control organisms were plotted against time
210 and fitted using simple linear regression. Statistical analyses of the data were
211 performed using 1-way analysis of variance (ANOVA) followed by the multiple
212 comparison test of Tukey (Zar 1996). The level of significance for statistical analyses
213 was always set at $\alpha = 0.05$.

214 A power analysis was performed using the whole set of data in order to assess the
215 minimal sample size of organisms (oysters and clams) required to detect realistic
216 (field-observed) differences in element concentration with statistical significance ($p <$
217 0.05) (Zar 1996).

218

219 **III. Results**

220 ***III.1. Experiment 1: Reciprocal transplantations***

221 *III.1.1. Sediments*

222 Comparison of element concentrations in sediments from the two stations naturally
223 inhabited by the oysters *I. isognomon* (Maa Bay and Boulari Bay) indicated that levels
224 of As, Co, Cr, Mn and Ni in sediments collected from Boulari Bay were significantly
225 higher ($p_{\text{Tukey}} \leq 0.0008$) than those collected from Maa Bay, whereas concentrations
226 of Cu and Zn were significantly higher ($p_{\text{Tukey}} \leq 0.0002$) in Maa Bay compared to
227 Boulari Bay (Table 2). No significant difference was observed between Cd
228 concentrations in sediments from the two bays.

229 Element concentrations measured in sediments from the two stations naturally
230 inhabited by the clams *G. tumidum* showed that concentrations of all elements in
231 sediments collected in Grande Rade (GR_{Int}, contaminated station,) were significantly
232 higher (p_{Tukey} always ≤ 0.0002) than those from Ouano Beach (clean station) (Table
233 2).

234 Element concentrations in sediments collected from the four stations at the different
235 times showed no significant variation with time.

236 *III.1.2. Oysters I. isognomon*

237 At the beginning of the experiment, concentrations of all elements in oysters from
238 Boulari Bay were significantly higher ($p_{\text{Tukey}} \leq 0.0002$, except for Zn: $p = 0.006$) than
239 those collected from Maa Bay, except for As, Cd and Mn for which no significant
240 difference was found.

241 Resident populations of *I. isognomon* from Maa Bay and Boulari Bay did not exhibit
242 any significant variation in concentrations of any element during the experiment time
243 course.

244 In oysters transplanted to the Boulari Bay station, the concentrations of Cr, Cu and Ni
245 showed a significant linear increase (k_u : 0.054, 0.065 and 0.031 $\mu\text{g g}^{-1} \text{d}^{-1}$; $p < 0.003$;
246 $R^2 = 0.14\text{-}0.24$) with time (Fig. 3). At the end of the experiment, Ni concentrations in
247 oysters were significantly lower ($p_{\text{Tukey}} = 0.046$) than those in resident oysters from
248 the Bay. No significant difference was found for Cr and Cu.

249 In oysters transplanted to the clean station (Maa Bay), only Ag, Co and Ni showed
250 significant depuration. Ag and Co concentrations showed a significant linear decrease
251 over time (k_e : 0.059 and 0.013 $\mu\text{g g}^{-1} \text{d}^{-1}$; $p < 0.03$; R^2 : 0.08 and 0.10, respectively;
252 Fig. 4). The depuration kinetics of Ni in oyster soft tissues was best fitted by an
253 exponential model (k_e : 0.19 d^{-1} , $R^2 = 0.54$, $p < 0.0001$). The concentrations of Ag, Co
254 and Ni in transplanted oysters at the end of the experiment were still significantly
255 higher (p_{Tukey} always ≤ 0.0001) than those in resident oysters.

256 *III.1.3. Clams G. tumidum*

257 At the beginning of the transplantation experiment (day 0), concentrations of all
258 elements in clams from Ouano Beach were significantly lower ($p_{\text{Tukey}} \leq 0.001$, except
259 for Mn and Zn, $p \leq 0.02$) than those from Grande Rade (GR_{Int}). The only exceptions
260 were As for which the highest concentration ($p_{\text{Tukey}} = 0.0003$) was measured in clams
261 from Ouano Beach, and for Cd for which no significant difference was found between
262 the clams of the two stations.

263 Control resident *G. tumidum* in Ouano Beach and Grande Rade showed no significant
264 variation for any element along the duration of the experiments.

265 In clams transplanted to the contaminated station (GR_{Int}), the concentrations of Ag,
266 Cd, Co, Cr, Cu and Zn displayed a significant linear increase (Ag, Cu and Zn k_u :
267 0.092, 0.105 and 0.21 $\mu\text{g g}^{-1} \text{d}^{-1}$, respectively; $p < 0.0001$; R^2 : 0.26 - 0.83; Cd, Co and
268 Cr k_u : 0.0014, 0.02 and 0.019 $\mu\text{g g}^{-1} \text{d}^{-1}$, respectively; $p < 0.02$; $R^2 \leq 0.12$) (Fig. 3).
269 The uptake kinetics of Ni in clam soft tissues was best fitted by an exponential model
270 ($R^2 = 0.65$, $p < 0.0001$) for which the estimated uptake rate constant, k_u , was 1.28 μg
271 $\text{g}^{-1} \text{d}^{-1}$. The uptake rate of Ag, Cu, Ni and Zn was higher by one order of magnitude
272 compared to that of the other elements (Fig. 3).

273 When clams from GR_{Int} were transplanted to the clean station, Ouano Beach, Ag and
274 As concentrations displayed a significant linear increase (k_u : 0.078 and 0.541 $\mu\text{g g}^{-1}$
275 d^{-1} ; $p < 0.001$; R^2 : 0.17 and 0.56, respectively) (Fig. 4). For the other elements, no
276 significant depuration was observed.

277 When a significant increase/decrease in element concentration was observed,
278 concentrations in transplanted organisms were compared to those of resident
279 organisms. Statistical analyses indicated that at the end of the experiment, Ag, Cd, Cr
280 and Cu concentrations in clams transplanted to GR_{Int} were significantly lower (p_{Tukey}
281 ≤ 0.005 , except for Ag, $p = 0.047$) than in resident clams from GR_{Int} (up to 3.9 fold
282 lower for Cd and Cr). No significant difference was found for Co, Ni and Zn
283 concentrations between transplanted and resident clams.

284 At the end of the experiment, Ag concentrations in clams transplanted to Ouano
285 Beach were significantly higher ($p_{\text{Tukey}} = 0.0001$) than those in resident clams at
286 Ouano Beach, whereas for As, the opposite was observed ($p_{\text{Tukey}} = 0.0003$).

287

III.2. Experiment 2: Transplantation in Grande Rade

288

III.2.1. Sediments

289 Sediments collected from Ouano Beach, Maa Bay, GR₁ and GR₂ revealed that
290 concentrations of all elements were significantly higher (1 to 3 orders of magnitude
291 higher) in sediments from GR₁ ($p_{\text{Tukey}} \text{ always } \leq 0.0002$) compared to the other three
292 stations, except for As that reached its highest concentration in GR₂ ($p_{\text{Tukey}} = 0.0002$)
293 (Table 2).

294

III.2.2. Oysters I. isognomon

295 Element concentrations in resident oysters from Maa Bay showed no significant
296 variation over the duration of experiment.

297 At the most contaminated station (i.e., GR₁), Co, Cr, Cu and Ni concentrations at 35
298 and 69 d were significantly higher than those at 0 d ($p_{\text{Tukey}} \leq 0.0006$ for Co, Cr, and
299 Cu and $p = 0.005$ for Ni; Fig. 5). Among these four metals, only Ni concentrations
300 after 69 d were significantly higher than those after 35 d of transplantation. Ag
301 concentration after 69 d was significantly higher than those at 0 d and after 35 d
302 ($p_{\text{Tukey}} = 0.03$), but no significant difference was found between concentrations at 0 d
303 and after 35 d of transplantation. Concentrations of As, Cd, Mn and Zn exhibited no
304 significant differences in the oysters at station GR₁ over the entire transplantation
305 period.

306 At station GR₂, which displays a lower degree of contamination than GR₁ according
307 to the element concentrations in sediments (Table 2), Ni concentrations after 35 and
308 69 d were significantly higher than those at 0 d and concentrations after 69 d were
309 significantly higher than those after 35 d ($p_{\text{Tukey}} \leq 0.0001$). Concentrations of Cr and
310 Cu after 35 and 69 d were significantly higher than those at 0 d ($p_{\text{Tukey}} \leq 0.0001$), but

311 no significant differences were found between 35 and 69 d. Ag concentrations after 69
312 d were significantly higher than those at 0 d ($p_{\text{Tukey}} = 0.0002$) and after 35 d ($p_{\text{Tukey}} =$
313 0.02), but no significant difference was found between concentrations at 0 d and after
314 35 d. No significant difference was found for the concentrations of As, Cd, Co, Mn
315 and Zn in oysters over the entire transplantation period in GR₂.

316 After 35 d, oysters transplanted into GR₁ displayed concentrations of Co, Cu and Ni
317 significantly higher than those at GR₂ ($p_{\text{Tukey}} \leq 0.0001$) whereas concentrations of Ag
318 and Zn in GR₁ oysters were significantly lower than those at GR₂ ($p_{\text{Tukey}} = 0.02$ and
319 0.048, respectively). After 69 d of transplantation, concentrations of Co, Cr, Cu, Mn
320 and Ni were significantly higher in oysters transplanted at station GR₁ than those at
321 GR₂ ($p_{\text{Tukey}} \leq 0.002$ for Co and Cu, and < 0.04 for Cr, Mn and Ni), whereas Ag
322 concentrations at GR₁ were significantly lower than those at GR₂ ($p_{\text{Tukey}} = 0.009$).

323 *III.2.3. Clams G. tumidum*

324 Element concentrations in resident clams from Ouano Beach showed no significant
325 difference over time.

326 At the most contaminated station (i.e., GR₁), Ag, Co and Ni concentrations after 35
327 and 69 d were significantly higher than those in clams measured at 0 d ($p_{\text{Tukey}} \leq$
328 0.0001 for Ni and ≤ 0.02 for Ag and Co) (Fig. 6) and concentrations after 69 d were
329 significantly higher than those after 35 d of transplantation. Concentrations of Cr and
330 Cu after 69 d were significantly higher than those at 0 and after 35 d ($p_{\text{Tukey}} \leq 0.0003$),
331 whereas no significant difference was found between the concentrations at 0 d and
332 after 35 d of transplantation. No significant difference was found between the
333 concentration of As, Mn and Zn after 35 and 69 d.

334 At the second station, GR₂, Ag and Ni concentrations after 35 and 69 d were
335 significantly higher ($p_{\text{Tukey}} \leq 0.0005$) than those at 0 d, and concentrations after 69 d
336 were significantly higher than those after 35 d ($p_{\text{Tukey}} = 0.0005$ and 0.03 respectively).
337 Cr concentrations after 35 and 69 d were significantly higher ($p_{\text{Tukey}} \leq 0.0001$) than
338 those at the beginning of the transplantation, but no significant differences were
339 observed between 35 and 69 d. Cu and Mn concentrations after 69 d of transplantation
340 were significantly higher ($p_{\text{Tukey}} = 0.039$ and 0.041) than those at the start of the
341 experiment. No significant difference was found for Co and Zn concentrations at 0,
342 and after 35 and 69 d. In contrast, As concentrations after 69 d were significantly
343 lower than those at day 0 ($p_{\text{Tukey}} = 0.014$).

344 Element concentrations after 35 and 69 d of transplantation were compared between
345 stations GR₁ and GR₂. Results indicated that after 35 d, Co, Cu and Ni concentrations
346 in clams at GR₁ were significantly higher than those at GR₂ ($p_{\text{Tukey}} \leq 0.0002$, except
347 for Cu: $p = 0.01$). For the other elements, no significant difference between GR₁ and
348 GR₂ was found after 35 d. After 69 d of transplantation, the concentrations of Cd, Co,
349 Cr, Cu and Ni in clams at GR₁ were significantly higher (p_{Tukey} always ≤ 0.0002) than
350 those at GR₂.

351 ***III.3. Estimation of the minimum sample size required to*** 352 ***detect a significant difference in concentrations***

353 A power analysis was performed to determine the minimum sample size necessary to
354 detect a significant difference ($\alpha = 0.05$) between concentrations of a given element in
355 two batches of clams or oysters. The variability of the data was shown to be
356 dependent upon the element, the species, the stations and the concentration levels.
357 The highest variance was observed in the samples displaying the highest

358 concentrations, consequently, minimum and maximum variance of the transplanted
359 batches were used to determine the range of minimal sample size necessary to detect
360 given differences of concentrations with statistical significance. Considered
361 differences of concentrations were selected to be representative of those that are
362 actually encountered in the field (Table 3). Generally, a sample of size ≥ 50 organisms
363 would be required to detect realistic differences in element concentrations, ranging
364 from 0.5 (Cd) to 150 (As) $\mu\text{g g}^{-1}$ dwt.

365 **IV. Discussion**

366 This field study investigated the *in situ* accumulation and depuration of 9 selected
367 elements in two tropical bivalves in order to validate their relevance as biomonitoring
368 species. Element concentrations in resident control organisms from each site showed
369 no significant variation with time during the transplantation time course, indicating
370 that any increase (or decrease) of element concentrations in tissues of the transplanted
371 individuals would actually reflect a higher (or a lower) metal contamination level at a
372 given site, and should not be due to seasonal factors.

373 When the oysters and clams from the clean sites were transplanted into the
374 contaminated sites (Experiment 1), the uptake of the selected elements displayed
375 different trends (Figs 3 and 4). At the end of the transplantation period, concentrations
376 observed in the organisms were either lower than or similar to those measured in
377 resident populations of the contaminated site, or did not change compared to their
378 initial levels.

379 Concentrations of Cr and Cu in oysters and Co, Ni and Zn in clams reached values
380 similar to those measured in resident organisms. Similar findings have been
381 previously reported for Cu and Zn in the soft tissues of the mussel *M. edulis*

382 transplanted to a temperate polluted bay (Roesijadi et al. 1984). However, since metal
383 uptake displayed linear kinetics over the transplantation period, the concentrations of
384 these elements would most probably have continued to increase if the duration of the
385 experiment was longer. This hypothesis is supported by the observations made in the
386 second transplantation experiment, in which clams transplanted to GR₁ and GR₂
387 displayed Co and Ni concentrations (up to 15.7 ± 4.8 and $140 \pm 46 \mu\text{g g}^{-1}$ dwt,
388 respectively) exceeding those of the resident clams from Grande Rade (7.2 ± 2.3 and
389 $63.2 \pm 13.5 \mu\text{g g}^{-1}$ dwt for Co and Ni, respectively).

390 In contrast, concentrations of Ni in transplanted oysters and, Ag, Cd, Cr, Cu in
391 transplanted clams significantly increased during the transplantation period but did
392 not reach the values measured in resident organisms. Taking into account the
393 measured uptake rate constants of these elements in oysters and clams, it can be
394 estimated that reaching the resident concentrations would require, for example, about
395 6 months for Ni in oysters and approximately 3 years for Cd in clams. Comparable
396 results have been previously reported for the oysters *Crassostrea rhizophorae*
397 (Wallner-Kersanach et al. 2000), the clam *Macoma balthica* (Cain and Luoma 1985)
398 and the mussel *M. edulis* from Greenland (Riget et al. 1997). However, our results
399 from the second transplantation (Experiment 2) indicated that when both species were
400 transplanted to a more contaminated site (GR₁), accumulation of Ni in oysters and Cr
401 in clams was faster than during the first transplantation experiment. Therefore, the
402 slow uptake rate of Ni in oysters and Cr in clams observed in the latter transplantation
403 is rather related to low bioavailability of these two metals at the contaminated site
404 (Boulari Bay and GR_{int} for oysters and clams, respectively) than to low
405 bioaccumulation efficiency of the organisms.

406 In the case of Ag, As, Cd, Co, Mn and Zn in oysters and As and Mn in clams,
407 concentrations did not show a significant increase during the transplantation from the
408 clean site to the polluted one (Experiment 1). Even though similar observations were
409 made for Cd and Zn concentrations in *Crenomytilus grayanus* after two months of
410 transplantation (Shulkin et al. 2003), opposite trends have also been observed. For
411 example, after 120 days of transplantation, a significant bioaccumulation of Cd and
412 Zn was measured in tissues of oysters, clams and cockles (Baudrimont et al. 2005).
413 Therefore, the lack of bioaccumulation of some elements in oysters and clams as
414 observed in our study suggests that these elements were rather poorly bioavailable for
415 the bivalves or that oysters and clams have efficient regulation mechanisms
416 preventing these metals from being accumulated. In fact, when organisms were
417 transplanted to GR₁ and GR₂ (Experiment 2), concentrations of Ag and Co in oysters
418 and Mn in clams were actually efficiently bioaccumulated. In addition, in laboratory
419 controlled conditions, metals including Co and Mn were efficiently accumulated in
420 oyster and clam tissues (Hédouin et al. 2010a). Therefore, these results support the
421 low bioavailability hypothesis, at least for Ag and Co in Boulari Bay and Mn at
422 Grande Rade GR_{Int}.

423 When organisms were transplanted to a clean station (Experiment 1), the
424 concentrations of all elements in both bivalves were almost the same after 100 d of
425 transplantation, except for Ag, Co and Ni in oysters, which showed a low but
426 significant decrease with time. However, Ag, Co and Ni concentrations in oysters
427 were far from reaching the concentrations measured in natural resident populations by
428 the end of the experiment. Such incomplete metal elimination has been reported by
429 several authors when organisms from polluted areas were transplanted to clean areas
430 (e.g., Zn in the mussel *Mytilus edulis*, Roesijadi et al. 1984, Simpson 1979; Cd and Cu

431 in the oyster *Crassostrea gigas*, Geffard et al. 2002; Cr, Cu and Zn in the clam
432 *Mercenaria mercenaria*, Behrens and Duedall 1981). The biological half life ($T_{b/2}$) of
433 these elements has been previously determined from radiotracer experiments in *I.*
434 *isognomon* and *G. tumidum* (Hédouin et al. 2007, 2010b). Although, elements like
435 Ag, Cd, Ni and Zn were very efficiently retained with $T_{b/2} \geq 5$ months, the other
436 elements displayed $T_{b/2}$ ranging from 1 to 3 months in both bivalve species,
437 independently of the uptake pathway tested (seawater, food or sediments).
438 Comparison of the data indicates that, in the field, depuration processes would take
439 longer for some metals than those previously estimated from laboratory experiments.
440 This confirms that laboratory results cannot always be extrapolated directly to
441 environmental situations, probably due to physiological adaptations of organisms
442 living in contaminated conditions (e.g. sequestration mechanisms). Since oysters and
443 clams showed very low depuration for most of the studied contaminants, bivalve
444 tissues would be able to retain information of contamination events over very long
445 periods of time. However, the subsequent drawbacks in a biomonitoring perspective
446 are that (1) the element concentrations in transplanted organisms are not actually able
447 to reflect the lower contamination levels occurring at a given location over a medium-
448 scale time period (i.e., 3 months), and (2) the element concentrations in organisms
449 collected from natural areas can reflect past contamination which is no longer
450 occurring rather than actual contamination. These drawbacks arise from the fact that
451 depuration is influenced by the past contamination history of the organisms. It was for
452 example shown that Cu was more easily eliminated (30% after 30 d) by oysters
453 temporarily transplanted into a metal-rich area for 60 d, than by resident oysters from
454 the same metal-rich area (decrease limited to 9% after 30 d) (Wallner-Kersanach et al.
455 2000). This suggests that our specimens from the more contaminated area, which

456 were exposed to high metal concentrations possibly for their whole life, may have
457 developed more efficient sequestering processes of metals to store them in their
458 tissues as non-toxic forms (e.g. in granules, Mason and Jenkins 1995). Such adaptive
459 mechanisms could occur in both studied species, and hence explain the efficient
460 retention observed in the field. Therefore, further studies should be focused on the
461 long-term depuration of elements in both bivalves from contaminated and clean sites,
462 in which bivalves would be previously exposed to contaminants in the field for 2-3
463 months before being transplanted into clean sites. Such experiments would
464 demonstrate whether the past contamination history of *I. isognomon* and *G. tumidum*
465 plays a role in the strong retention of elements observed in the field.

466 Interestingly, when clams from Grande Rade (GR_{Int}) were transplanted to Ouano
467 beach (Experiment 1), a significant bioaccumulation of As was observed in clam
468 tissues, although lower As concentration was reported in sediments from Ouano beach
469 (3.1 µg g⁻¹ dwt). High level of As in clam tissues from Ouano beach has been recently
470 reported (Hédouin et al. 2009), and the authors suggested that food was the main
471 pathway of As uptake in clams. Our transplantation experiment from Grande Rade
472 (GR_{Int}) to Ouano beach showed that As was highly bioavailable for clams in Ouano
473 beach. In addition, due to the low levels of As in sediments from Ouano beach, this
474 result supports the assumption that the high levels of As are most probably
475 bioaccumulated from the diet of the organisms (Sanders et al. 1989, Warnau et al.
476 2007, Hédouin et al. 2009). Since the clam *G. tumidum* is a seafood product in New
477 Caledonia and that its tissues showed high levels of As, the sources of As in Ouano
478 Beach and the potential toxicity of As for consumers should be further investigated.

479 In the second transplantation (Experiment 2), element concentrations in sediments
480 clearly indicated that GR₁ is the most contaminated site, reaching very high level of

481 Co, Cr, Mn, and Ni (up to 10,500 $\mu\text{g Ni g}^{-1}$ dwt). These high concentrations in metals,
482 and more specifically in Ni, concur with the very high concentration of Ni observed in
483 the particulate phase within the water column (Migon et al. 2007). Ag, Co, Cr, Cu and
484 Ni were efficiently accumulated in transplanted oysters and clams. In addition, results
485 indicate that bioaccumulation was dependent on sampling location and species, and
486 difference in the contamination level of the two stations was easier to observe when
487 organisms were transplanted for a longer time (69 vs 35 d). For example, our results
488 showed that the concentrations of 5 elements in bivalve tissues (Co, Cr, Cu, Mn and
489 Ni in oysters and Cd, Co, Cr, Cu and Ni in clams) were significantly higher at GR₁
490 than at GR₂ after 69 d, whereas differences were significant only for 3 elements (Co,
491 Cu and Ni) after 35 d.

492 In this second transplantation experiment, oysters and clams were transplanted to the
493 same stations, hence exposed to the same environmental conditions. Their
494 bioaccumulation capacities can thus be directly compared. Clams were more efficient
495 than oysters in bioaccumulating the selected elements (e.g., concentrations measured
496 after 69 d of transplantation increased by a factor 7 in oysters and by a factor 40 in
497 clams). These findings were surprising considering previous results from laboratory
498 radiotracer studies (e.g., Hédouin et al. 2010a) which indicated a more efficient
499 bioconcentration capacity in oysters than in clams when exposed to dissolved
500 elements (concentration factors were higher by several orders of magnitude). Such a
501 difference between laboratory and *in situ* experiments strongly suggests that the
502 seawater pathway is not the major route of accumulation driving global metal uptake
503 in these organisms. Rather, ingestion of particulate materials would be the main
504 pathway for metal uptake, an hypothesis that is supported by a previous study of Cd,
505 Co and Zn bioaccumulation modeling in *I. isognomon* and *G. tumidum* (Hédouin

506 2006, 2010b). This may indeed explain the higher metal levels in *G. tumidum* which
507 lives buried in the sediment, and feeds mainly on organic (and metal)-rich particles at
508 the seawater-sediment interface.

509 Combining the results from transplantations 1 and 2 demonstrated the usefulness of
510 bioindicator species to assess the degree of contamination present in the marine
511 environment. Indeed, for some elements, the high levels of metals reported in
512 sediments were reflected in organism tissues (e.g. Cr, Ni) and a significant
513 bioaccumulation of these metals was observed in the tissues of the clams and the
514 oysters during the transplantation experiments. However, for some elements, the
515 metal bioaccumulation trends observed in clams and oysters were different from those
516 expected based on metal concentrations found in sediments at the different sites of
517 transplantation. For example, in sites characterized with low As concentrations in
518 sediments (Ouano beach), efficient bioaccumulation of As was observed in clam
519 tissues (Experiment 1), suggesting that other sources of As uptake are available for
520 organisms (e.g. food, see discussion above). In contrast, for Mn, although high levels
521 were measured in sediments, almost no bioaccumulation was observed in organism
522 tissues (Experiment 2). This clearly points out that only a fraction of the metals
523 present in the sediments is bioavailable for organisms. Mn bound in the lattice of
524 naturally occurring Mn-rich ores (e.g., laterite and garnierite) may be less available
525 for uptake by marine organisms compared to water-soluble forms. The different
526 patterns of metal bioaccumulation observed in clams and oysters during the two
527 transplantation experiments carried out in this work pointed out that the metal
528 contamination status cannot be based solely on metal analysis from the sediments and
529 this is the reason why the use of bioindicator species is an important asset to better
530 characterize the contamination status of a particular site. In addition, although it was

531 not performed in the present study, metal analysis in seawater is also a useful
532 complementary information to those obtained from sediments and organisms.
533 However accurate analysis of metals in seawater is uneasy and expensive, and is
534 therefore generally not integrated in biomonitoring programmes. Nevertheless,
535 nowadays the development of techniques such as the diffusive gradients in thin films
536 (DGT) (e.g., Davison and Zhang 1994, Webb and Keough 2002) brings new insights
537 to obtain time-integrated information on metal concentration in seawater. Ideally
538 analysis of metals in sediments, seawater and organisms will be recommended for
539 biomonitoring purposes since such combination enhances our understanding of the
540 contamination status present in the marine environment, but also brings additional
541 information for identifying the source of contamination.

542 In order to obtain accurate and reliable data in biomonitoring programmes, the
543 determination of optimal sample size to be collected is of fundamental importance. In
544 this context, the present study has investigated the minimum sample size required to
545 detect a given difference in concentration. Results shown in Table 3 indicate that the
546 detection of a $0.5 \mu\text{g g}^{-1}$ dwt difference in tissue concentrations in the highly
547 contaminated organisms required the largest sample size. Relatively large variability
548 in metal concentrations in organisms within a site has frequently been reported (e.g.,
549 Daskalakis 1996, Gordon et al. 1980). In the present study, the concentration
550 variability was higher with increasing average concentration. Consequently, detecting
551 small differences in concentration among organisms with higher metal concentrations
552 will require an increase in sample size. Nevertheless, it is important to keep in mind
553 that to be feasible, the sample size required in a biomonitoring programme should
554 always remain realistic.

555 Compared to the actual metal concentration range measured in the New Caledonia
556 lagoon waters and sediments, the minimum difference in concentrations detectable
557 with sample sizes of 50-60 organisms would allow for an efficient differentiation
558 among sites naturally inhabited by the two targeted bivalves. For example, a
559 difference of $2 \mu\text{g Ni g}^{-1}$ dwt can be detected with a sample size of 7 oysters and 36
560 clams in a population showing low Ni levels (Table 3). However, 62 oysters and 30
561 clams would be necessary to detect differences of 30 and $8 \mu\text{g g}^{-1}$ dwt, respectively, in
562 a population characterized by high Ni concentrations (Table 3). A sample size of 50-
563 60 organisms was similarly recommended by other authors in order to facilitate the
564 detection of significant changes in concentrations (e.g. Gordon et al. 1980, Topping
565 1983). In current biomonitoring programmes, organisms collected (20 oysters and 30
566 mussels for the NOAA Mussel Watch, Beliaeff et al. 1998; 10 oysters and 50 mussels
567 for the French RNO, Claisse 1989) are pooled before analysis in order to reduce costs
568 of sample preparation and analysis. However, pooling leads to the loss of statistical
569 information on inter-individual variability, which is obviously an important issue to
570 assess significance of concentration differences among samples. These economic
571 constraints are obvious in the case of large national and international biomonitoring
572 programmes that assess the levels of numerous trace elements and organic
573 contaminants in many stations. However, in New Caledonia, which is mainly
574 impacted by mining activities, metal and metalloids are the contaminants of major
575 concern. Therefore, analytical costs would be reduced compared to biomonitoring
576 programmes that include the very expensive analysis of organic compounds. Hence,
577 in the specific context of the New Caledonia lagoon, it is highly recommended to
578 analyze individual samples in order to obtain information on inter-individual

579 variability that would provide scientifically-supported best practices in environmental
580 management.

581 **V. Conclusion**

582 This study clearly indicates that the clam *G. tumidum* can be recommended for an
583 active monitoring of contaminants in subtidal and intertidal stations of the New
584 Caledonia lagoon on a spatiotemporal scale. Biomonitoring studies using transplanted
585 organisms would be an efficient solution to survey environmental levels of key local
586 metal contaminants in areas lacking resident bivalves. The advantage of using
587 transplanted organisms (active biomonitoring) over sampling resident populations
588 (passive biomonitoring) is that it allows selecting organisms of uniform initial
589 element concentrations, of common origins and past history, and thus ensures
590 comparable biological samples. However, if further studies confirm the observed very
591 long element retention times in these organisms, organisms from sites displaying a
592 low contamination will have to be used in order to prevent bias in element
593 concentrations due to physiological adaptation of organisms (e.g. sequestration
594 mechanisms).

595

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606

607 **References**

608 Baudrimont, M., Schafer, J., Marie, V., Maury-Brachet, R., Bossy, C., Boudou, A.
609 and Blanc, G. (2005) Geochemical survey and metal bioaccumulation of three
610 bivalve species (*Crassostrea gigas*, *Cerastoderma edule* and *Ruditapes*
611 *philippinarum*) in the Nord Medoc salt marshes (Gironde estuary, France). *Science*
612 *of the Total Environment* 337, 265-280.

613 Behrens, W.J. and Duedall, I.W. (1981) The behaviour of heavy metals in
614 transplanted hard clams, *Mercenaria mercenaria*. *Journal du Conseil International*
615 *pour l'Exploitation de la Mer* 39, 223-230.

616 Beliaeff, B., O' Connor, T.P. and Claisse, D. (1998) Comparison of chemical
617 concentrations in mussels and oysters from the United States and France.
618 *Environmental Monitoring and Assessment* 49, 87-95.

619 Bird, E.C.F., Dubois, J.P. and Iltis, J.A. (1984) The impacts of opencast mining on the
620 rivers and coasts of New Caledonia, Tokyo, Japan, 53 p.

621 Boyden, C.R. (1977) Effect of size upon metal content of shellfish. *Journal of the*
622 *Marine Biological Association of the United Kingdom* 57, 675-714.

623 Breau, L. (2003) Etude de la bioaccumulation des métaux dans quelques espèces
624 marines tropicales: recherche de bioindicateurs de contamination et application à la
625 surveillance de l'environnement côtier dans le lagon sud-ouest de la Nouvelle-
626 Calédonie, University of La Rochelle La Rochelle, France, 282 p.

627 Cain, D.J. and Luoma, S.N. (1985) Copper and silver accumulation in transplanted
628 and resident clams (*Macoma balthica*) in South San Francisco Bay. *Marine*
629 *Environmental Research* 15, 115-135.

630 Claisse, D. (1989) Chemical contamination of French coasts : the results of a ten years
631 Mussel Watch. *Marine Pollution Bulletin* 20, 523-528.

632 Daskalakis, K. (1996) Variability of metal concentrations in oyster tissue and
633 implications to biomonitoring. *Marine Pollution Bulletin* 32(11), 794-801.

634 Davison, W. and Zhang, H. (1994) *In situ* speciation measurements of trace
635 components in natural waters using thin film gels. *Nature* 367, 546-548.

636 Geffard, A., Amiard, J.-C. and Amiard-Triquet, C. (2002) Kinetics of metal
637 elimination in oysters from a contaminated estuary. *Comparative Biochemistry and*
638 *Physiology C Comparative Pharmacology & Toxicology* 131(3), 281-293.

639 Goldberg, E.D., Koide, M., Hodge, V., Flegal, A.R. and Martin, J.H. (1983) U.S.
640 Mussel Watch: 1977-1978 results on trace metals and radionuclides. *Estuarine,*
641 *Coastal and Shelf Science* 16, 69-93.

642 Gordon, M., Knauer, G.A. and Martin, J.H. (1980) *Mytilus californianus* as a
643 bioindicator of trace metal pollution: variability and statistical considerations.
644 *Marine Pollution Bulletin* 11(7), 195-198.

645 Hédouin, L. (2006) Caractérisation d'espèces bioindicatrices pour la surveillance des
646 activités minières et la gestion de l'environnement en milieu récifal et lagunaire:
647 application au lagon de Nouvelle-Calédonie, University of La Rochelle, La
648 Rochelle, France, 328 p.

649 Hédouin, L., Metian, M., Teyssié, J.-L., Fowler, S.W., Fichez, R. and Warnau, M.
650 (2006) Allometric relationships in the bioconcentration of heavy metals by the
651 edible tropical clam *Gafrarium tumidum*. *Science of the Total Environment* 366(1),
652 154-163.

653 Hédouin, L., Pringault, O., Metian, M., Bustamante, P. and Warnau, M. (2007) Nickel
654 bioaccumulation in bivalves from the New Caledonia lagoon: seawater and food
655 exposure. *Chemosphere* 66, 1449-1457.

656 Hédouin, L., Bustamante, P., Fichez, R. and Warnau, M. (2008) The tropical brown
657 alga *Lobophora variegata* as a bioindicator of mining contamination in the New
658 Caledonia lagoon: a field transplantation study. *Marine Environmental Research*
659 66(4), 438-444.

660 Hédouin, L., Bustamante, P., Churlaud, C., Pringault, O., Fichez, R. and Warnau, M.
661 (2009) Trends in concentrations of selected metalloid and metals in two bivalves
662 from the coral reefs in the SW lagoon of New Caledonia. *Ecotoxicology and*
663 *Environmental Safety* 72, 372-381.

664 Hédouin, L., Gomez Batista, M., Metian, M., Buschiazzi, E. and Warnau, M. (2010a)
665 Metal and metalloid bioconcentration capacity of two tropical bivalves for
666 monitoring the impact of land-based mining activities in the New Caledonia
667 lagoon. *Marine Pollution Bulletin*, 61, 554-567.

668 Hédouin, L., Metian, M., Teyssié, J.-L., Fichez, R. and Warnau, M. (2010b)
669 Delineation of heavy metal uptake pathways (seawater, food, and sediment) in
670 tropical marine oysters using radiotracers. *Marine Pollution Bulletin*, 61, 542-553.

671 Mason, A.Z. and Jenkins, K.D. (1995) Metal speciation and bioavailability in aquatic
672 systems. Tessier, A. and Turner, D.R. (eds), pp. 479-608, John Wiley & Sons,
673 Chichester.

674 Metian, M. (2003) Bioaccumulation des métaux lourds chez 4 espèces marines du
675 lagon de Nouvelle Calédonie: Caractérisation de leur potentiel bioindicateur pour
676 le monitoring des activités minières locales, Master Thesis, IAEA-MEL, Monaco /
677 Université Libre de Bruxelles, Belgium, 44 p.

678 Metian, M., Bustamante, P., Hédouin, L. and Warnau, M. (2008a) Accumulation of
679 nine metals and one metalloid in the tropical scallop *Comptopallium radula* from
680 coral reefs in New Caledonia. *Environmental Pollution* 152(3), 543-552.

681 Metian, M., Giron, E., Borne, V., Hédouin, L., Teyssié, J.-L. and Warnau, M. (2008b)
682 The brown alga *Lobophora variegata*, a bioindicator species for surveying metal
683 contamination in tropical marine environments. *Journal of Experimental Marine*
684 *Biology and Ecology* 362(1), 49-54.

685 Migon, C., Ouillon, S., Mari, X. and Nicolas, E. (2007) Geochemical and
686 hydrodynamic constraints on the distribution of trace metal concentrations in the
687 lagoon of Nouméa, New Caledonia. *Estuarine, Coastal and Shelf Science* 74(4),
688 756-765.

689 Phillips, D.J.H. (1991) Selected trace elements and the use of biomonitors in
690 subtropical and tropical marine ecosystems. *Reviews of Environmental*
691 *Contamination and Toxicology* 120, 105-128.

692 Phillips, D.J.H. and Rainbow, P.S. (1993) *Biomonitoring of trace aquatic*
693 *contaminants*, London, 371 p.

694 Riget, F., Johansen, P. and Asmund, G. (1997) Uptake and release of lead and zinc by
695 blue mussels. Experience from transplantation experiments in Greenland. *Marine*
696 *Pollution Bulletin* 34(10), 805-815.

697 Roesijadi, G., Young, J.S., Drum, A.S. and Gurtisen, J.M. (1984) Behaviour of trace
698 metals in *Mytilus edulis* during a reciprocal transplant field experiment. *Marine*
699 *Ecology Progress Series* 18, 155-170.

700 Sanders, J.G., Osman, R.W. and Riedel, G.F. (1989) Pathways of arsenic uptake and
701 incorporation in estuarine phytoplankton and the filter-feeding invertebrates

702 *Eurytemora affinis*, *Balanus improvisus* and *Crassostrea virginica*. Marine
703 Biology 103, 319-325.

704 Shulkin, V.M., Presley, B.J. and Kavun, V.I. (2003) Metal concentrations in mussel
705 *Crenomytilus grayanus* and oyster *Crassostrea gigas* in relation to contamination
706 of ambient sediments. Environment International 29(4), 493-502.

707 Simpson, R.D. (1979) Uptake and loss of zinc and lead by mussels (*Mytilus edulis*)
708 and relationships with body weight and reproductive cycle. Marine Pollution
709 Bulletin 10(3), 74-78.

710 Topping, G. (1983) Guidelines for the use of biological material in first order
711 pollution assessment and trend monitoring Scottish Fisheries Research Report, N.
712 (ed), Department of Agriculture and Fisheries for Scotland, Marine Laboratory, 28
713 p.

714 Wallner-Kersanach, M., Theede, H., Eversberg, U. and Lobo, S. (2000) Accumulation
715 and elimination of trace metals in a transplantation experiment with *Crassostrea*
716 *rhizophorae*. Archives of Environmental Contamination and Toxicology 38(1), 40-
717 45.

718 Warnau, M., Gomez-Batista, M., Alonso-Hernandez, C., Regoli, F., (2007) Arsenic: is
719 it worth monitoring in the Mediterranean Sea?. In: Marine Sciences and Public
720 Health - Some Major Issues CIESM Workshop Monographs No. 31, pp. 83-86
721 (Monaco).

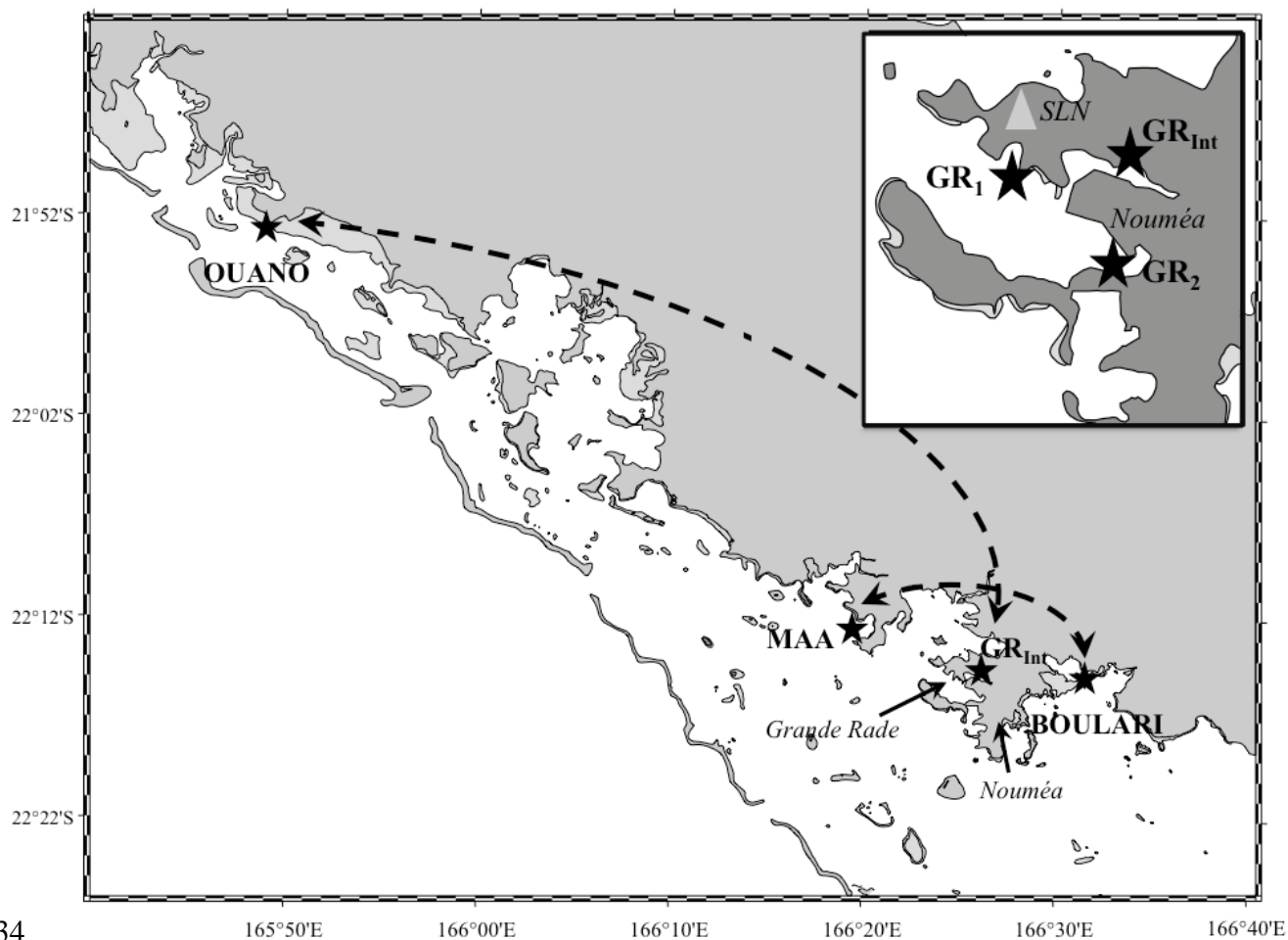
722 Webb, J.A. and Keough, M.J. (2002) Measurement of environmental trace-metal
723 levels with transplanted mussels and diffusive gradients in thin films (DGT): a
724 comparison of techniques. Marine Pollution Bulletin 44(3), 222-229.

725 Whicker, F.W. and Schultz, V. (1982) Radioecology: nuclear energy and the
726 environment, CRC Press, Boca Raton, Florida, USA, 320 p.

727 Zar, J.H. (1996) Biostatistical analysis, 3rd ed. Prentice-Hall, Upper Saddle River,
728 New Jersey, 662 p.

729

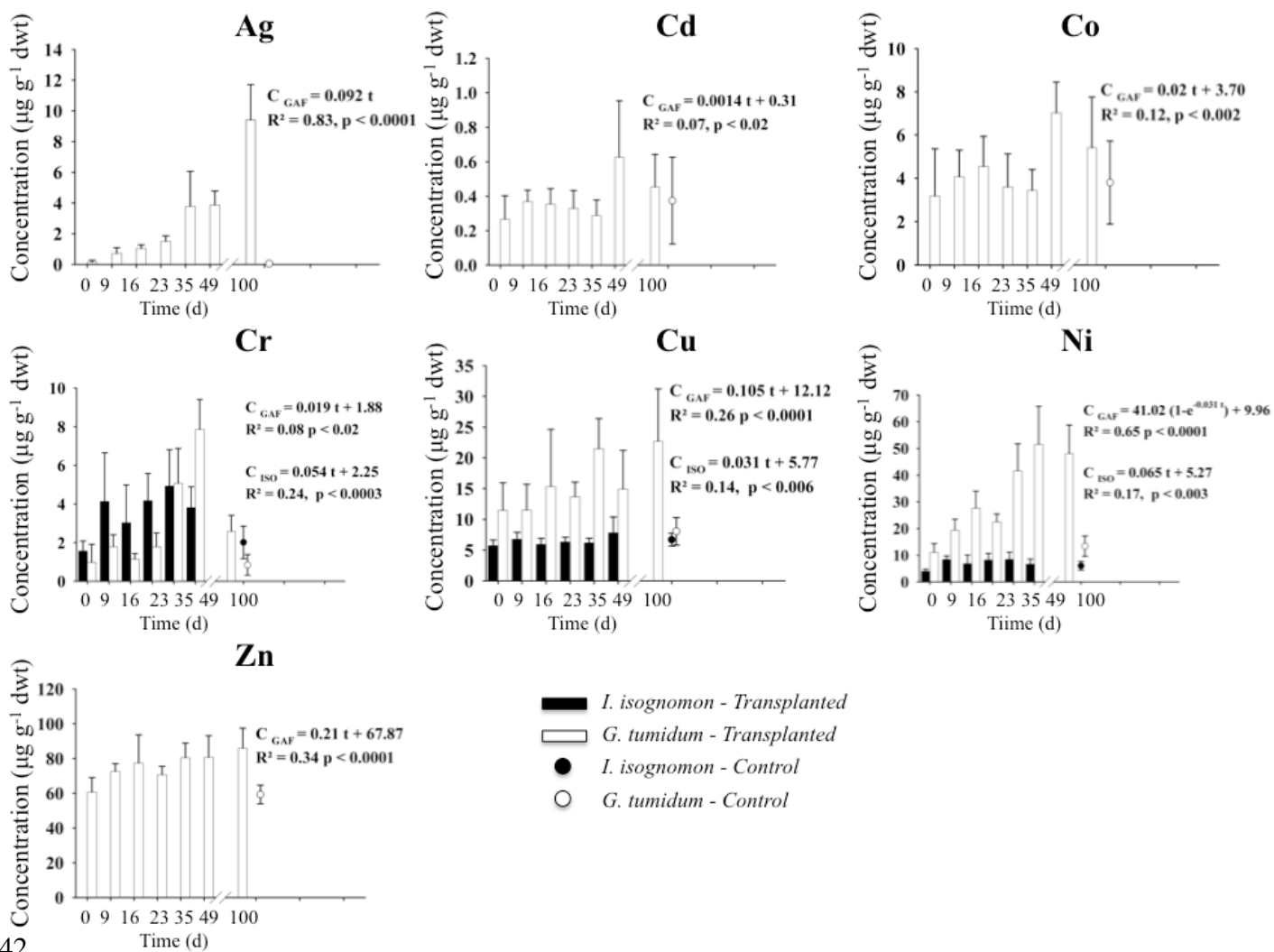
730 **Figure 1.** Map showing the stations selected for transplantation experiments.
731 OUANO: Ouano Beach; MAA: Maa Bay; BOULARI: Boulari Bay; GR_{Int}: Grande
732 Rade Interdital station; GR₁: Grande Rade subtidal site 1; GR₂: Grande Rade subtidal
733 site 2; SLN : « Société Le Nickel » Nickel ore processing plant.



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736 **Figure 2.** Element concentrations (mean \pm SD; $\mu\text{g g}^{-1}$ dwt; $n = 7$ for transplanted
 737 organisms and $n = 5$ for control organisms) in oysters *Isognomon isognomon* and
 738 clams *Gafrarium tumidum* transplanted from clean stations, Maa Bay (*I. isognomon*)
 739 and Ouano Beach (*G. tumidum*), to the contaminated stations, Boulari Bay and
 740 Grande Rade (GR_{Int}), respectively.

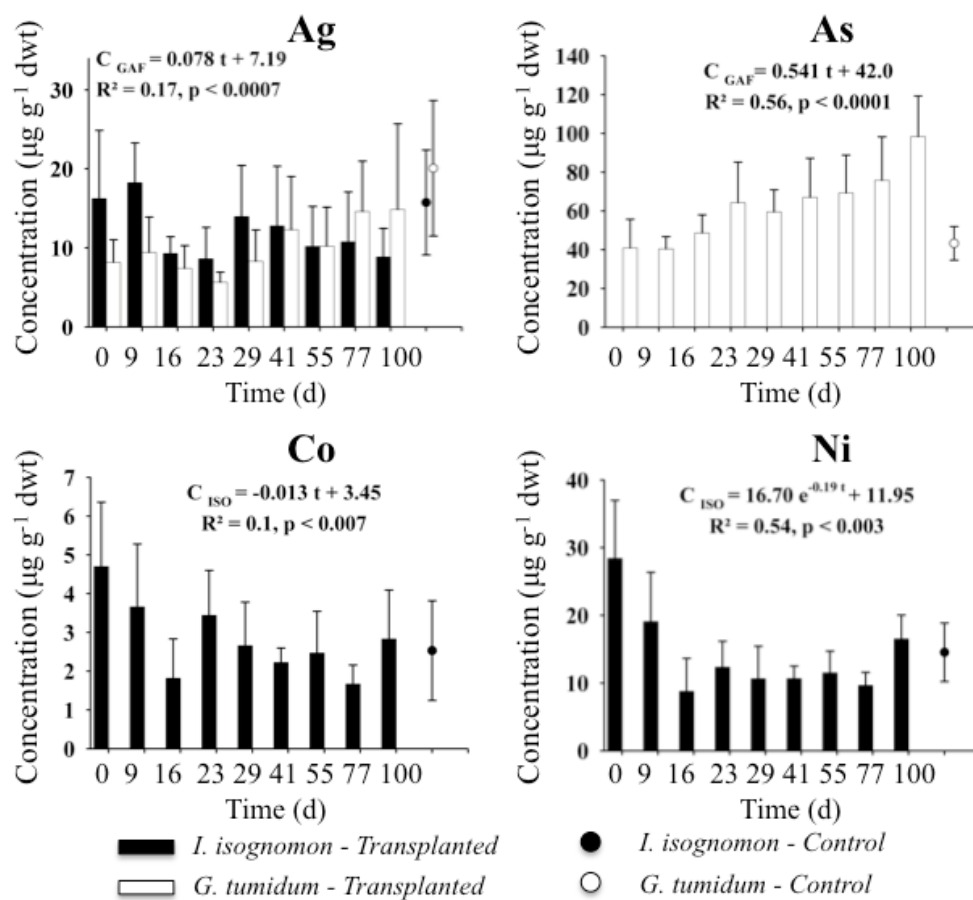
741 (only data showing a significant regression, $p < 0.05$, are presented)



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744 **Figure 3.** Element concentrations (mean \pm SD; $\mu\text{g g}^{-1}$ dwt; $n = 7$ for transplanted
 745 organisms and $n = 5$ for control organisms) in oysters *Isognomon isognomon* and
 746 clams *Gafrarium tumidum* transplanted from the contaminated stations, Boulari Bay
 747 (*I. isognomon*) and Grande Rade (GR_{Int}, *G. tumidum*), to reference stations, Maa Bay
 748 and Ouano Beach, respectively. (only data showing a significant regression, $p < 0.05$,
 749 are presented)



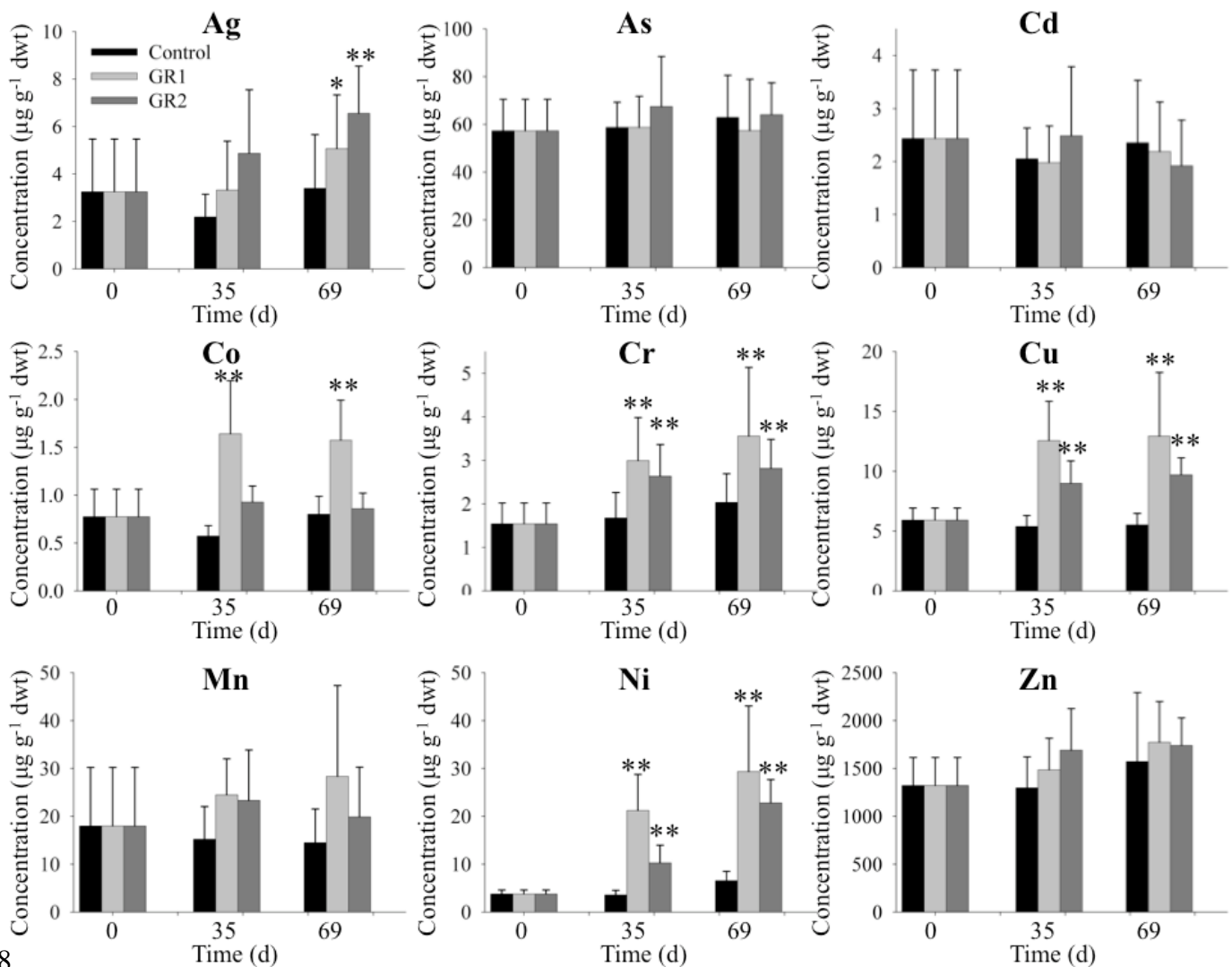
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752 **Figure 4.** Element concentrations (mean \pm SD; $\mu\text{g g}^{-1}$ dwt; n = 30 for transplanted
 753 organisms and n = 20 for control organisms) in oysters *Isognomon isognomon* from Maa
 754 Bay transplanted into stations GR₁ and GR₂ in the Grande Rade.

755 (stars indicate that the concentration is significantly different from those in organisms
 756 at 0 d; * p < 0.05, ** p < 0.001)

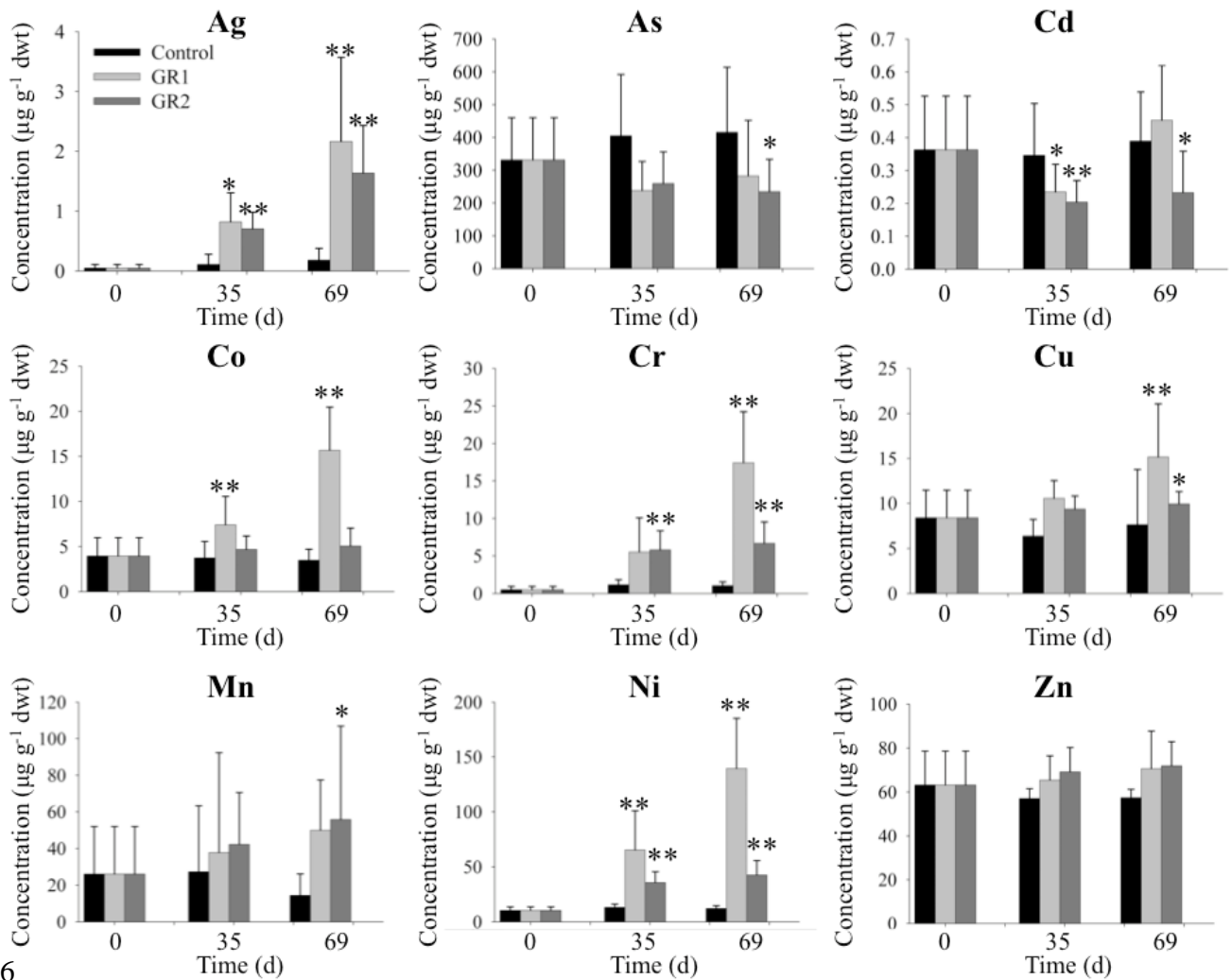
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760 **Figure 5.** Element concentrations (mean \pm SD; $\mu\text{g g}^{-1}$ dwt; $n = 30$ for transplanted
 761 organisms and $n = 20$ for control organisms) in clams *Gafrarium tumidum* from
 762 Ouano Beach transplanted into the stations GR₁ and GR₂ in the Grande Rade.
 763 (stars indicate that the concentration is significantly different from those in organisms
 764 at 0 d; * $p < 0.05$, ** $p < 0.001$)

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769 **Table 1.** ICP-OES and ICP-MS analysis of certified reference materials: certified values and measured values (mean \pm SD $\mu\text{g g}^{-1}$ dwt)

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Element	Method	TORT-2			DOLT-3		
		Found <i>Mean \pm SD</i>	Certified <i>Mean \pm SD</i>	% Recovery	Found <i>Mean \pm SD</i>	Certified <i>Mean \pm SD</i>	% Recovery
Ag	ICP-MS	No certified value			1.07 \pm 0.092	1.20 \pm 0.07	89.3
As	ICP-OES	22.28 \pm 2.22	21.60 \pm 1.80	103.2	9.45 \pm 0.97	10.20 \pm 0.50	92.7
Cd	ICP-MS	26.42 \pm 3.75	26.70 \pm 0.60	99.0	17.01 \pm 3.12	19.40 \pm 0.60	87.7
Co	ICP-MS	0.52 \pm 0.089	0.51 \pm 0.091	101.5	No certified value		
Cr	ICP-OES	0.66 \pm 0.19	0.77 \pm 0.15	85.3	No certified value		
Cu	ICP-OES	98.40 \pm 11.17	106.0 \pm 10.0	92.8	31.23 \pm 2.40	31.20 \pm 1.00	100.1
Mn	ICP-OES	12.46 \pm 1.19	13.60 \pm 1.20	91.6	No certified value		
Ni	ICP-OES	2.02 \pm 0.35	2.50 \pm 0.19	80.9	3.05 \pm 0.76	2.72 \pm 0.35	112.1
Zn	ICP-OES	187.6 \pm 19.6	180.0 \pm 6.0	104.2	97.67 \pm 6.97	86.60 \pm 2.40	112.8

774 **Table 2.** Element concentrations (mean \pm SD; $\mu\text{g g}^{-1}$ dwt, n = 3) in sediments collected in six sampling sites.

775 GR_{Int}: Grande Rade Intertidal station; GR₁: Grande Rade subtidal site 1; GR₂: Grande Rade subtidal site 2

	Ouano beach	Maa bay	Boulari bay	GR _{Int}	GR ₁	GR ₂
Ag	0.019* \pm 0.028	0.013* \pm 0.014	0.06* \pm 0.04	0.35* \pm 0.13	0.17* \pm 0.09	0.018* \pm 0.015
As	3.1* \pm 1.2	6.4* \pm 0.3	16.7* \pm 1.3	8.0* \pm 1.2	7.0* \pm 5.9	15.4 \pm 0.5*
Cd	0.4 \pm 0.2	1.0 \pm 0.2	1.1 \pm 0.3	2.5 \pm 0.2	3.7 \pm 1.2	0.8 \pm 0.1
Co	0.8 \pm 0.4	4.4 \pm 2.3	15.4 \pm 11.1	49.2 \pm 5.2	366 \pm 145	6.1 \pm 0.9
Cr	7.8 \pm 2.4	46.9 \pm 4.0	71.5 \pm 10.2	309 \pm 39	1,290 \pm 410	24.6 \pm 2.9
Cu	1.4* \pm 0.7	7.0 \pm 0.5	0.9* \pm 0.1	27.0 \pm 3.6	9.6 \pm 3.3	2.8* \pm 0.4
Mn	44.7 \pm 14.9	134 \pm 6.7	545 \pm 53.0	304 \pm 15	1,600 \pm 600	76.7 \pm 8.1
Ni	5.6 \pm 3.0	69.2 \pm 5.6	101 \pm 12.9	848 \pm 78	10,500 \pm 3,300	66.4 \pm 15.8
Zn	3.5 \pm 2.0	16.3 \pm 1.3	7.1 \pm 1.6	148 \pm 11.0	73.3 \pm 22.7	12.8 \pm 1.8

776 *: inferior to detection limit.

777 **Table 3.** Minimal sample size of the oyster *Isognomon isognomon* and the clam *Gafrarium*
 778 *tumidum* necessary to detect with 90 % significance a difference ($p < 0.05$) of concentrations
 779 between two groups of organisms.

780 Observed range of element concentrations represents concentrations that have been
 781 measured in the two species resident from different stations along the New Caledonia coast;
 782 number between brackets represents concentrations that have been reached during
 783 transplantation experiments.

Element	Species	Observed Concentration range in tissues*	Difference ($\mu\text{g g}^{-1}$ dwt)	Sample size (number of individuals required)			
				<i>I. isognomon</i>		<i>G. tumidum</i>	
				Low	High	Low	High
Ag	Oyster	1.5 - 32.8	1	21	110	< 3	43
			3	4	14	< 3	6
	Clam	0.02 - 33.1	10	< 3	< 3	< 3	< 3
			30	< 3	< 3	< 3	< 3
As	Oyster	21.6 - 76.6	10	32	111	3,713	8,260
			20	9	29	921	2,065
	Clam	37.4 - 441	40	4	8	231	517
			80	< 3	< 3	59	130
			150	< 3	< 3	18	38
350	< 3	< 3	5	8			
Cd	Oyster	1.2 - 2.5	0.2	220	894	4	160
			0.5	36	144	< 3	27
	Clam	0.17 - 1.8	1	10	37	< 3	8
			2	4	10	< 3	4
Co	Oyster	0.5 - 2.5	0.2	8	170	780	> 10,000
			0.5	< 3	28	126	1,945
	Clam	1.1 - 7.2 (15.7)	1	< 3	8	32	487
			2	< 3	< 3	9	122
			5	< 3	< 3	< 3	21
10	< 3	< 3	< 3	6			
Cr	Oyster	1.6 - 9.0	1	9	54	7	993
			2	4	15	< 3	248
	Clam	1.1 - 10.5 (17.4)	4	< 3	5	< 3	63
			8	< 3	< 3	< 3	17
15	< 3	< 3	< 3	8			
Cu	Oyster	3.1 - 17.3	2	6	153	15	184
			4	< 3	39	5	47
	Clam	5.6 - 88.2	8	< 3	11	< 3	12
			15	< 3	4	< 3	5
			30	< 3	< 3	< 3	< 3
60	< 3	< 3	< 3	< 3			
Mn	Oyster	17.0 - 34.7	2	260	1,938	727	8,590
			4	66	485	183	2,148
	Clam	5.5 - 187.4	8	18	122	47	538
			15	6	36	14	154
			30	< 3	10	5	40
60	< 3	4	< 3	11			
120	< 3	< 3	< 3	4			
Ni	Oyster	2.2 - 16.0 (32.4)	2	7	963	36	6,505
			4	< 3	242	10	1,627
	Clam	8.1 - 63.2 (140)	8	< 3	62	4	410
			15	< 3	19	< 3	117
			30	< 3	5	< 3	30
60	< 3	< 3	< 3	9			
120	< 3	< 3	< 3	4			
Zn	Oyster	1700 - 13,820	5	> 10,000	> 10,000	14	248
			10	722	4,180	5	63
	Clam	55.6 - 154	50	182	1,045	< 3	4
			100	9	43	< 3	< 3
			500	4	12	< 3	< 3
10,000	< 3	< 3	< 3	< 3			

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* from Breau 2003, Hédouin et al. 2008a, Present study